

WHAT IS CLAIMED IS:

1. A method for reducing or eliminating cooling injury in living systems, comprising:

selecting a tonicity of nonpenetrating components of a solution within an optimum range for minimizing cooling injury;

preparing a preservation medium at said optimum tonicity of the nonpenetrating components, wherein said preservation medium comprises one or more cryoprotectants sufficient in concentration to induce vitrification upon cooling;

adding said preservation medium to a living system; and

cooling the living system to a temperature below about 0°C.

2. The method of Claim 1, wherein the optimum tonicity of the nonpenetrating components is hypertonic.

3. The method of Claim 2, wherein the optimum tonicity is from 1 to 4 times isotonic.

4. The method of Claim 2, wherein the optimum tonicity is from 1.1 to 2.7 times isotonic.

5. The method of Claim 2, wherein the optimum tonicity is from 1.1 to 2 times isotonic.

6. The method of Claim 2, wherein the optimum tonicity is from 1.2 to 1.5 times isotonic.

7. The method of Claim 1 wherein the optimum tonicity is established by raising the concentration of the carrier solution for the cryoprotective agents.

8. The method of Claim 7, wherein the tonicity of the carrier solution for the cryoprotective agents is raised simultaneously with an increase in concentration of the cryoprotective agents.

9. The method of Claim 7, wherein the tonicity of the carrier solution for the cryoprotective agents is increased in similar relative proportion to the increase in the concentration of the cryoprotective agents.

10. The method of Claim 9, further comprising:

returning the tonicity of the carrier solution to isotonic while the concentration of the cryoprotective agents is diluted in similar proportion to the dilution of the carrier solution.

5 11. A method for the rapid addition of cryoprotective agents to a cell or tissue or organ, comprising:

adding an amount of cryoprotectant under isotonic conditions; and
adding a second amount of the cryoprotectant in one step with a simultaneous increase in the concentration of the carrier solution or of other impermeants such that the overall tonicity of the medium is increased by a similar relative proportion to the increase in
10 concentration of the cryoprotectant.

12. The method of Claim 11, wherein the cryoprotectant is rapidly removed by simultaneous dilution of the cryoprotectant and impermeants, said dilution representing a similar proportional change in the concentrations of the cryoprotectant and of the impermeants of the solution.

15 13. A method for the prevention of cooling injury, comprising:
optimizing a vitrification solution by adding antinucleating polymers in concentrations that increase the tonicity of the medium to within the optimal range for inhibition of cooling injury.

20 14. The method of Claim 13, wherein the antinucleating polymers are selected from the group consisting of: polyglycerol, polyvinyl alcohol-polyvinyl acetate copolymer and a mixture thereof.

15. The method of Claim 13, wherein the tonicity is optimized using polyvinyl pyrrolidone of mean molecular mass 5000 daltons or polyethylene glycol of mean molecular mass 1000 daltons.

25 16. The method of Claim 13, wherein said vitrification solution comprises: dimethyl sulfoxide, formamide, and ethylene glycol.

30 17. The method of Claim 14, wherein said vitrification solution comprises dimethyl sulfoxide, formamide, and ethylene glycol, polyglycerol, and polyvinyl alcohol-polyvinyl acetate copolymer, and wherein the combination of polyglycerol and polyvinyl alcohol-polyvinyl acetate copolymer is at a total concentration of 0.1 to 0.7 times isotonic.

18. The method of Claim 16 wherein the vitrification solution further comprises acetol.

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